TABLE 26

OCT4/PPIA	on seeding	day 7 (24 well)	day 14 (24 well)
sample 6	0.52	4.43	3.44
sample 16	0.52	1.7	0.93
sample 17	0.52	1	0.56
sample 18	0.52	2.27	0.74

Experimental Example 16: Proliferation, Maintenance of Survival, Dispersibility of Cell Aggregates of HEK293-IFNβ Cells in 3D Culture Using Mixture of Chitin Nanofiber and Chitosan Nanofiber, Mixture of Chitin Nanofiber and Chitosan Nanofiber Produced by Simultaneous Fibrillating, or Poly(1,4)-N-Acetyl-β-D-Glucosamine Nanofiber Having Specific Acetylation Degree

[0180] A medium composition was prepared by adding the sample 1 obtained above to EMEM medium (manufactured by Wako Pure Chemical Industries, Ltd.) containing 10% (v/v) fetal bovine serum to a final concentration of 0.02% (w/v) or 0.1% (w/v). In addition, a medium composition was prepared by adding sample 6, 14 or 15 to EMEM medium (manufactured by Wako Pure Chemical Industries, Ltd.) containing 10% (v/v) fetal bovine serum to a final concentration of 0.02% (w/v). Furthermore, a no addition medium composition (sample 12) which does not contain the above-mentioned base material was prepared. The final concentrations of each nanofiber in the prepared nanofiber-containing EMEM medium containing 10% (v/v) fetal bovine serum were as the follows:

[0181] (1) Medium composition containing sample 1 (chitin nanofiber:0.02% (w/v), chitosan nanofiber:0% (w/v))

[0182] (2) Medium composition containing sample 1 (chitin nanofiber:0.1% (w/v), chitosan nanofiber:0% (w/v))

[0183] (3) Medium composition containing sample 6 (chitin nanofiber:0.02% (w/v), chitosan nanofiber:0.08% (w/v))

[0184] (4) Medium composition containing sample 14 (chitin/chitosan nanofiber prepared simultaneous fibrillating:0.1% (w/v)

[0185] (5) Medium composition containing sample 15 (nanofiber with adjusted amount of N-acetylglucosamine (50%):0.1% (w/v))

[0186] Successively, HEK293-IFMβ cells that produce cultured human IFMβ stably were suspended in each of the above-mentioned medium compositions at about 200000 cells/mL, and seeded in 24 well flat bottom ultra low attachment surface microplate (manufactured by Corning Incorporated, #3473) at 1.2 mL/well. The cells were cultured in a CO_2 incubator (37° C., 5% CO_2) in a static state for 21 days at maximum, and the medium was changed every 1 or 2 days. On seeding (day 0) and days 7, 14, 21 after seeding, each culture medium was suspended and 300 μL was dispensed. An ATP reagent (300 μL, CellTiter-Glo™ Luminescent Cell Viability Assay, manufactured by Promega) was added and suspended, and the suspension was stood for about 10 min at room temperature. The luminescence intensity (RLU value) was measured by FlexStation3 (manufactured by Molecular Devices) and the luminescence value of the medium alone was subtracted to calculate the number of viable cells. On days 8, 15, 21 after seeding, photographs were taken and dispersibility of cell sphere clumps was confirmed.

[0187] As a result, proliferation and survival maintenance effect were found under all conditions with the addition of each base material as compared with no addition conditions. When a medium composition containing chitin/chitosan nanofiber prepared by simultaneous fibrillating or nanofiber with adjusted amount of N-acetylglucosamine was used, a cell density higher than that by chitin nanofiber alone was found. By time-course observation, a large cell aggregate was found on day 8 with no addition, cell aggregates gradually grew even with chitin nanofiber alone (sample 1), and the number of aggregates decreased. It was considered that further coagulation of cell aggregates occurred under these conditions. On the other hand, when a medium composition containing chitin nanofiber and chitosan nanofiber or a medium composition containing chitin/chitosan nanofiber prepared by simultaneous fibrillating was used, coagulation of cell aggregates was suppressed. Particularly, when a medium composition containing nanofiber with adjusted amount of N-acetylglucosamine was used, small cell aggregates tended to be dispersed even on day 21. The results of viable cells number are shown in FIG. **1**, and the results of photograph observation are shown in FIG. **2** (day 8), FIG. **3** (day 15), and FIG. **4** (day 21).

INDUSTRIAL APPLICABILITY

[0188] According to the present invention, high-quality adherent cells (e.g., mesenchymal stem cells) can be produced efficiently. When the cells obtained by the present invention are stem cells, they are very high-quality stem cells that maintain properties such as undifferentiated state, chemotacticity and the like in a preferable state. Therefore, the stem cells obtained by the present invention (e.g., mesenchymal stem cells) can be preferably used to supplement organs and tissues lost due to diseases and the like. In addition, the cell secretion products obtained by the present invention can also be used in the treatment of various diseases. Therefore, the present invention is considered to be extremely useful in, for example, the field of regenerative medicine.

[0189] This application is based on a patent application No. 2018-164042 filed in Japan (filing date: Aug. 31, 2018) and a patent application No. 2019-134058 filed in Japan (filing date: Jul. 19, 2019), the contents of which are incorporated in full herein.

1. A medium composition for suspension culture of an adherent cell, comprising

(1) a chitin nanofiber; and

(2) a chitosan nanofiber or a polysaccharide.

2. The medium composition according to claim **1**, wherein the medium composition comprises the chitin nanofiber and the chitosan nanofiber, and a ratio of the chitin nanofiber and the chitosan nanofiber is chitin nanofiber: chitosan nanofiber=1:0.5-20.

3. The medium composition according to claim **1**, wherein the medium composition comprises the chitin nanofiber and the polysaccharide, and the polysaccharide is selected from the group consisting of methylcellulose, deacylated gellan gum, and sodium alginate.

4. The medium composition according to claim **1**, wherein the adherent cell is a cell that self-aggregates under suspension culture.